

IN THE CLAIMS

C¹ 6. (Three times amended) The method according to Claim 23, wherein the gene encoding said [native] indigenous protease has been deleted by homologous or illegitimate recombination.

7. (Three times amended) The method according to Claim 23, wherein a plasmid comprises said expression cassette [DNA sequence].

C² 10. (Three times amended) The method according to Claim 23, wherein at least one copy of said expression cassette [DNA sequence] is integrated into the genome of said host.

C³ 11. (Twice amended) The method according to Claim 10, wherein said host further contains at least one copy of a plasmid comprising said [DNA sequence] expression cassette.

C⁴ 12. (Twice amended) A method of obtaining an alkalophilic *Bacillus* strain having no detectable [a reduced] extracellular high alkaline protease [level], said method comprising:

transforming an alkalophilic *Bacillus* strain with a cloning vector comprising the 5' and the 3' flanking regions but not the coding region of gene coding for [a] the high alkaline protease and wherein a sufficient amount of said flanking regions is present to provide for homologous recombination with an indigenous gene coding for [a] the high alkaline protease whereby transformants are obtained;

growing said transformants under conditions whereby the replication function encoded by said vector is inactivated; and

isolating transformants identified as having [a reduced] no detectable extracellular high alkaline protease [level].

C⁵ 14. (Twice amended) An alkalophilic *Bacillus* strain producing a mutant high alkaline protease substantially free of expression product of an indigenous extracellular

C5 emil.
alkaline protease gene, wherein said strain has been obtained by transforming an [mutant] alkalophilic *Bacillus* strain having [a reduced] no detectable indigenous extracellular high alkaline protease [level] obtained by the method according to Claim 12 or 13 with a plasmid expression vector comprising [a] the mutant high alkaline protease gene.

C6
17. (Twice amended) A ~~mutant~~ high alkaline protease produced according to the method of Claim 23 and characterized as (1) substantially free from contamination with an indigenous extracellular high alkaline protease, and (2) differing in at least one amino acid from the indigenous [a wild-type] high alkaline protease. 102 produced by process

C7
23. (Amended) A method for production of a [mutated] mutant high alkaline protease substantially free of indigenous extracellular high alkaline protease, said method comprising:

growing an alkalophilic *Bacillus* ~~strain host~~ having [a reduced] no detectable indigenous extracellular high alkaline protease [level] as a result of deletion of the gene for said indigenous extracellular protease transformed with an expression cassette providing for expression of [a mutated] said mutant high alkaline protease in said host, whereby said [mutated] mutant high alkaline protease is produced.

C8
26. (Amended) A method for production of a [mutated] mutant high alkaline protease substantially free of indigenous extracellular protease, said method comprising:

growing an asporogenous *Bacillus* strain host having [a reduced] no detectable indigenous extracellular protease [level] as a result of deletion of the gene for said indigenous extracellular protease transformed with an expression cassette providing for expression of [a mutated] said mutant high alkaline protease in said host, whereby said [mutated] mutant high alkaline protease is produced.

REMARKS

The Invention

The claimed invention is directed to methods and compositions for preparation of mutant high alkaline proteases and mutant alkalophilic *Bacillus* strains which produce only the mutant high alkaline protease and not the corresponding indigenous protease. Also claimed are a detergent composition comprising as an active ingredient one or more high alkaline proteases prepared according to the claimed method and use of the high alkaline protease in a detergent composition or a laundry process.

Pending Claims

Prior to the entry of the above amendments, Claims 4-7, 9-17, 19 and 23-26 are pending. Claims 23, 4-7, 9-11, and 26 are directed to methods for production of a mutant high alkaline protease; Claims 12-13 are directed to a method of obtaining an alkalophilic *Bacillus* strain having a reduced extracellular alkaline protease level; Claims 14-16 are directed to an alkalophilic *Bacillus* strain producing a mutant high alkaline protease; Claim 17 is directed to mutant a high alkaline protease; Claim 19 is directed to a detergent composition comprising as an active ingredient a alkaline protease. Claim 24 is directed to a method of preparing a detergent composition comprising an alkaline protease as active ingredient. Claim 25 is directed to a method of processing laundry with the claimed detergent composition.

The Office Action

The specification was objected to under 35 USC § 112, first paragraph, as failing to provide an enabling disclosure of a source for *Bacillus* novo species PB92. Claims 4-7, 9-17, 19 and 23-26 were rejected under 35 USC § 112, first paragraph, for the reasons set forth in the objection to the specification. Claims 12, 14, 17, 23, 26 and their dependent claims 4-7, 9-11, 19, and 24-25 were rejected under 35 USC § 112, first paragraph, on the basis that they are not limited to methods of producing an alkalophilic asporogenic *Bacillus* novo species PB92 of minimal indigenous extracellular protease level, transformed with a mutated B. novo PB92 alkaline protease. Claims 2, 9, 12, 14, and 17-21 were rejected under 35 USC § 112, second paragraph, as indefinite. Claims 4-7, 9-17, 19, and 23-26 were rejected under 35 USC § 103, as unpatentable over Fahnestock et al. and Estell et al. in view of TeNijenhuis and Suggs et al.

These rejections are addressed individually below.

Amendments

Claims 12, 14, 23 and 26 have been amended to recite that the *Bacillus* strain has no detectable indigenous high alkaline protease. Claim 12 has also been amended to make clear that the transforming vector contains the 5' and 3' flanking regions but not the coding region of the high alkaline protease gene. Claim 17 has been amended to make clear that the claimed mutant protease differs by at least one amino acid from the indigenous protease. Claims 23 and 26 have been amended to recite "mutant" rather than "mutated" to reflect the language of Claims 14 and 17. Claims 6, 7, 10 and 11 have been amended to provide proper antecedent basis for the language of the Claims. No new matter is introduced by the above amendments, and the Examiner is respectfully requested to enter them.

Section 112, first paragraph

The specification was objected to and claims 4-7, 9-17, 19 and 23-26 were rejected under 35 USC § 112, first paragraph, for failing to provide an enabling disclosure regarding the source of the *B. novo* species PB92 strain or DNA from said strain. This rejection is respectfully traversed.

The Examiner has stated that the specification describes the manipulation of the protease gene from strain PB92, but does not describe a way of obtaining such DNA, or a source of the DNA or strain. *Bacillus novo* species PB92 strain has been deposited with the American Type Culture Collection and has been assigned the ATCC number 31408. A Declaration of Biological Deposit accompanies this response. This *Bacillus* strain is well known in the art, having been the subject of U.S. Patent No. Re. 30602, reissued May 5, 1981. The methods for obtaining the DNA from this strain are well known in the art. See, for example, EPA 0284126.

Claims 12, 14, 17, 23, 26, and their dependent claims 4-7, 9-11, 19 and 24-25 were rejected under 35 USC § 112, first paragraph, on the basis that they are not limited to methods of producing an alkalophilic asporogenic *Bacillus novo* species PB92 of minimal indigenous extracellular protease level, transformed with a mutated *B. novo*

species PB92 alkaline protease. This rejection is in part avoided by amendment and in part respectfully traversed.

The Examiner has stated that Claims 12, 14, 23 and 26 are not properly enabled for the recitation of "reduced extracellular alkaline protease levels" and "a gene coding for a high alkaline protease," that the claims are not properly enabled for the recitation of the phrase "mutant high alkaline protease," that claim 17 is not enabled for such proteases "differing in at least one amino acid from a wild-type high alkaline protease," that the rejected claims are not properly enabled for any and all "alkalophilic *Bacillus*" strains, and that the rejected claims are not properly enabled for the term "reduced."

The claims now recite that the *Bacillus* strains have "no detectable indigenous extracellular high alkaline protease" or "no detectable indigenous extracellular protease". Support for this amendment is found in the specification of page 17, line 18 through page 18, line 15. The specification clearly teaches methods for the selection and characterization of protease negative *Bacillus* strains. Claim 17 now recites that the claimed mutant protease differs from "the indigenous" protease rather than "a wild-type" protease.

Applicants disagree that the claims are not properly enabled for a "gene coding for a high alkaline protease," for "mutant high alkaline protease," for "differing in at least one amino acid" and for any and all "alkalophilic *Bacillus*." The present invention lies in the discovery by the inventors that *Bacillus* strains could be made that have a lower level of protease by deleting a protease gene by a double homologous recombination. These *Bacillus* strains are then particularly suitable for the production of a mutant protease introduced by transformation with the mutant gene. This invention was exemplified with and is preferably carried out with alkalophilic *Bacilli*, particularly *B. novo* species PB92, deleted for the indigenous high alkaline protease gene and transformed with a mutant high alkaline protease gene to produce a mutant high alkaline protease free from contamination with the indigenous high alkaline protease. Having the teaching of the present invention, it would be within the skill of one of ordinary skill in the art to obtain *Bacillus* strains having a reduced level of protease by deleting a particular protease gene according to the method of the invention. From the teaching of the present application, one of ordinary skill in the art would understand that a *Bacillus* protease gene could be deleted by

introducing a vector having the 5' and 3' flanking regions but not the coding region of the protease gene. The specification teaches how to select and characterize the protease negative transformants on pages 17-18. This invention is exemplified using the gene for the high alkaline protease of *Bacillus novo* species PB92, but one of ordinary skill in the art would recognize that the invention could be practiced with any alkalophilic *Bacillus*, or indeed any *Bacillus* having a protease gene. Applicants wish to point out that *B. licheniformis*, *B. amyloliquefaciens* and *B. subtilis* are not alkalophilic *Bacillus* species. The definition for alkalophilic *Bacilli* is given in the specification on page 10, lines 19-33. Although not taxonomically well classified, these *Bacillus* species are well known in the art. renew

The specification provides examples of mutant high alkaline proteases on page 12, line 38 through page 13, line 3, in the Examples on page 23, lines 19-23 and in Table 1 on page 29. Mutant high alkaline proteases are well known in the art. The specification provides detailed procedures for producing mutant protease genes on page 21, line 32 through page 23, line 1. It would be well within the skill of one of ordinary skill in the art to determine which mutations would result in a protease differing by at least one amino acid from the indigenous protease, least one amino acid from the indigenous protease. No, not for effects.

In view of the above remarks and the amendments to the claims, the claims are now clearly enabled by the specification and the Examiner is respectfully requested to withdraw this rejection.

Section 112, second paragraph

Claims 2, 9, 12, 14, and 17-21 were rejected under 35 USC § 112, second paragraph, as being indefinite. This rejection is in part avoided by amendment and in part respectfully traversed.

This Office Action repeats many of the same reasons for rejection made in the Office Action mailed October 24, 1991. Claims 9, 14, 17 and 19 were amended to avoid the rejection and Claims 2, 18 and 21-22 were canceled in the response to the previous Office Action submitted on April 24, 1992. The only new reason for rejection is that given for Claim 12.

Claim 12 was rejected as not being clear from the claim language where the gene for the indigenous protease is located within the cloning vector. Claim 12 has been amended to recite that the cloning vector comprises the 5' and the 3' flanking regions but not the coding region of a gene coding for the high alkaline protease.

Claim 14 was rejected as vague and indefinite for recitation of the phrase "capable of producing". The phrase "capable of" was deleted in the amendment submitted on April 24, 1992. Claim 17 was rejected as being unclear as to which alkaline protease the claim intended. Claim 17 was amended to avoid this rejection in the amendment submitted on April 24, 1992. Claim 18 was rejected for improperly reciting "*Bacillus* strain". Claim 18 was canceled in the amendment submitted on April 24, 1992. Claims 19-21 were rejected as confusing and/or incorrect in the recitation of "proteases according to Claim 16". Claim 19 was previously amended to the singular "protease". Claims 20-21 were canceled.

Section 103

Claims 4-7, 9-17, 19 and 23-26 were rejected under 35 USC § 103 as being unpatentable over Fahnestock et al. and Estell et al. in view of TeNijenhuis and Suggs et al. This rejection is respectfully traversed.

Fahnestock et al. disclose the inactivation of a protease gene by insertion of a cloned CAT gene; the present invention discloses the inactivation by deletion of an indigenous extracellular protease gene. Insertion of a CAT gene in an otherwise complete protease gene makes possible the reversion of the inactivation of the protease, whereas in the strain according to the claimed invention, the gene is deleted and reversion is therefore not possible. Further, claims 12-14 specifically relate to deletion of genes using homologous or illegitimate recombination using the flanking regions of the gene to be deleted, a concept not taught by Fahnestock et al. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

Estell et al. disclose the production of mutated serine and neutral protease using *B. subtilis* as a host. This is not an alkalophilic *Bacillus* strain. Furthermore, the protease deficient strain *B. subtilis* BG84 used to express mutant protease was made protease deficient by NTG mutagenesis. The inactivating mutation of the neutral protease

gene consisted of a deletion of 527 bp BSA1 fragment. A large part of the neutral protease gene, including a part of the coding region, therefore remained intact. Estell et al. does not teach the use of alkalophilic *Bacilli* having completely deleted protease encoding genes. ?

TeNijenhuis disclosed the presence of an alkaline protease in media containing *B. novo* species PB92. TeNijenhuis does not disclose the sequence of this protein nor the sequence of the gene encoding it. Furthermore, TeNijenhuis does not suggest the use of a recombinant thereof. Finally, no suggestion is made of the preferred use of alkalophilic *Bacilli* for production of high alkaline proteases.

Suggs et al. is merely a general description of how to perform mutagenesis. The present application does not claim mutagenesis, only a method for producing mutant proteins.

Prior to the present invention, alkalophilic *Bacillus* strains had not been reported to be useful in protease production, due in part to the fact that they are difficult to transform. In addition, it was thought that *Bacillus* strains used for protease production must be sporulating. This teaching is found in Estell et al. who stress that *Bacillus* should preferably be normally sporulating. Thus, it was not obvious at the time of the present invention that asporogenic *Bacillus* having little or no high alkaline protease could be made or that once made, would be suitable for the production of mutant proteases.

Conclusion

In view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the under signed attorney at (415) 494-7622.

Respectfully submitted,

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